both direct retroviral gene transfer as well as adenoviral gene transfer.

During vascular isolation, we first injected a concentrated solution of a retroviral vector containing human heat-stable alkaline phosphatase into skeletal muscle 4 days after lidocaine-induced regeneration and angiogenesis. In these experiments we documented a substantially higher expression of recombinant protein than that achieved using the previously described technique of cell-mediated gene therapy. Recombinant gene expression was observed in both the muscle and capillary endothelium.

This technique of vascular isolation of the hind limb has also been used to test the transduction efficiency of adenoviral vectors. Current gene therapy strategies using adenoviral vectors targeted to the lung or liver have been complicated by an inflammatory response and an associated tissue injury. We hypothesized that adenoviral transduction of rat skeletal muscle capillary bed during vascular isolation would produce gene transfer sufficient to achieve systemic levels of a recombinant protein without tissue injury. During vascular isolation, rats received a replication-incompetent adenovirus (Ad) encoding for either the marker gene, human placental alkaline phosphatase (hpAP) or interleukin-1 receptor antagonist (IL-1ra), an antagonist of IL-1 mediated disease. Gene transfer over a $10^9 \times 10^{12}$ particle/mL range to the gastrocnemius capillary endothelium and muscle fibers was highly efficient and titer dependent, reaching a maximum transduction rate in these tissues of 71% ± 7% and 25% ± 5%, respectively, 5 days after gene transfer. Adenoviral-induced inflammation in skeletal muscle was not observed, and distant organ transfer was minimal or absent. Gastrocnemius muscle from rats given Ad-IL-1ra had 241 ± 66 pg IL-1ra/mg protein, whereas the Ad-hpAp negative control had 35 ± 14 pg IL-1-ra/mg protein. More importantly, in the Ad-IL-1ra transduced rats, a serum level of 185 ± 20 pg/mL IL-1ra was documented. Athymic rats given Ad-IL-1ra had serum levels of 493 ± 62 pg/mL IL-1ra 14 days after transduction, an IL-1ra was detected for up to 98 days. Serum from Ad-IL-1ra athymic rats significantly inhibited IL-1 β-induced (1 ng/mL) PGE_2 production from cultured endothelial cells by 82% ± 2%. Thus this gene transfer strategy results in substantial transduction of endothelium and muscle fibers, sufficient to achieve pharmacologic levels of IL-1ra, without evidence of tissue injury.

We believe the capillary bed of skeletal muscle continues to hold considerable promise as a technique of somatic gene therapy to deliver recombinant proteins to the systemic circulation, such as human factor VIII or IX deficiency in hemophiliacs or for the purposes of treating vascular diseases such as the induction of angiogenesis in a chronic ischemic limb.

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CELL-BASED GENE TRANFER IN BIOMATERIALS

A number of biomaterials in a wide variety of configurations and materials have been adapted to gene transfer technology. Special needs such as immunoisolation, size of the gene product molecule, biodegradability, cell carrying capacity, and durability both of the material and gene expression determine physical characteristics. Hollow fibers, pillows, and microspheres are commonly used. With a single gene the null mutant apolipoprotein deficient (knockout) mouse converts a species highly resistant to atherosclerosis to one that is highly susceptible. This mutant model develops severe atherosclerosis on a normal diet. The lesions closely resemble human lesions.

Using a plasmid containing the cDNA encoding apo E, fibroblasts or mouse mammary tumor cells, and two constructs: (1) retroviral vectors based on LNL6 retroviral vector (MoMLV), the long terminal repeat promoter (LTR) and one of several enhancers; or (2) a recombinant bovine papillomavirus expression vector containing a mouse metallothionein I promoter, the human metallothionein IA gene and, in both, the human apo E gene, we have been able to transfect the fibroblasts and mouse mammary tumor cells, load the cells into the devices named above, and obtain apo E expression both in tissue culture and in the plasma of mice into the cell-loaded devices implanted. Coincident with the appearance of apo E in the mouse plasma was a substantial reduction in serum cholesterol and triglycerides. Inhibition of atherosclerosis throughout the aorta of the apo E–deficient mouse was prominent in the treated mouse compared with the untreated control, (28% vs 69%, respectively, based on planimetric measurement of the lesions visible on the surface of the aortas).

This is an excellent animal model in which to study atherogenesis and its progression and regression. As far as therapeutic potential, there are several obstacles and pitfalls, including a low level of transfected gene expression, eventual loss of gene expression, need for continuing replication of the transfected cells, escape of implanted cells from the device, induction of host viremia, and unpredictable consequences of random gene insertion.

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SMOOTH MUSCLE CELLS FOR SYSTEMIC AND LOCAL THERAPY

Smooth muscle cells are an excellent target for vascular gene transfer because they have many properties that make them a reliable and durable vector. They are the principle cellular component of large arteries and veins and participate in various important vascular disease states, including atherosclerosis and intimal hyperplasia, after vascular reconstruction. The cells are readily cultured and, as we have shown, can be seeded back in vivo. These seeded cells, like their endogenous relatives, are long-lived. We have used vascular smooth muscle cells transduced with replication-defective retrovirus as a means for transferring genes into blood vessels for three purposes: (1) systemic gene therapy; (2) local pharmacology to control vascular function; and (3) the development of models of vascular disease. This method of transferring genes leads to stable, long-term expression of the virally encoded protein because the transferred gene is incorporated into the host genome. A significant weakness in this approach is that cell culture and subsequent seeding back into the artery are required for efficient gene transfer. Because transduction with these vectors is so poor in vivo (approximately 1 in 10,000 cells), the only efficient way to transfer the gene of interest is to transduce the cells in vitro and then to transfer the cells to the vascular site. The cells are placed into a discrete portion of the vasculature (e.g., an injured artery or a graft) and can be removed later by surgical excision.[1-3] Although there has been neither detectable helper virus activity nor evidence of neoplastic transformation in the smooth muscle cells we have studied in vivo, it is reassuring to have a practical means to deal with the problem should the need arise.

The expression of the transferred gene is controlled at the level of transcription by the retroviral LTRs and is continuous and not regulatable. This limitation of the system is not of great significance because the clinical problem is to obtain adequate expression of the transferred gene and not to suppress overexpression. Nevertheless, in the future we hope to have mechanisms for the control of the expression of the transferred gene.

Systemic gene therapy

Most of our studies have been performed in syngeneic Fischer 344 rats using retroviral vectors developed by Miller and colleagues.[1,4] These retroviral vectors do not contain the necessary components to encode for the coat proteins and therefore are replication-defective. The regulatory elements (LTRs) remain, and the gene of interest together with a selectable marker such as neomycin phosphotransferase (NEO) are inserted. The complete vector is generated by transfecting the retroviral plasmid containing the gene of interest into a "packaging line" that provides the necessary proteins for encapsulating the genetic material. The viral particles generated in this manner act as one-way shuttles to infect vascular smooth muscle cells. As noted above, they are not capable of replication. In the initial experiments, the Fischer 344 rat smooth muscle cells were infected with retrovirus encoding either *E. coli* beta galactosidase (β gal) or human placental alkaline phosphatase.[1,2] The transduced cells are selected in the neomycin analog G418. The cells stably expressing either β gal or alkaline phosphatase are then seeded into rat carotid arteries denuded of endothelium by the passage of a balloon catheter. The smooth muscle cells stick in regions lacking endothelium within 15 minutes and remain after the circulation is restored. Three major issues have been addressed. First, it appears that the cultured smooth muscle cells are robust and can survive culture, retroviral infection, and harsh selection in vitro and still survive long term in the in vivo environment. Second, these cells infected with retrovirus do not appear to be transformed. They replicated for one or two rounds in the neointima and then resume quiescence in a manner similar to the endogenous cells. An intima formed by seeded cells reaches maximal thickness between 2 and 4 weeks after surgery and does not change thereafter up to 1 year. Third, the expression of the transferred genes appears to be durable. We have evaluated the expression of a number of human and animal genes (β gal, human alkaline phosphatase, human adenosine deaminase, baboon tissue inhibitor of metalloproteinases-1 [TIMP-1], rat erythropoietin), and have found that the encoded proteins are expressed and active, although in some circumstances an immunologic response to the expressed protein has been observed (for example, to baboon TIMP-1).

We have been able to demonstrate recently that the encoded protein can have a systemic impact. In rats seeded with smooth muscle cells encoding rat erythropoietin, the hematocrit levels rise from 45% to 60% over a period of several weeks[5] even though endogenous erythropoietin expression is suppressed in these animals. Although this systemic effect can be obtained with relatively few cells (approximately 10,000 to 50,000 cells per carotid artery), a note of caution is in order. Erythropoietin is an extremely potent hormone, and a very small amount produces a dramatic response. Either more effective vectors or larger numbers of smooth muscle cells would be required to achieve expression of a protein in the microgram-per-milliliter range.

Models of vascular disease

We are currently using the smooth muscle cell–based transfer approach to model certain aspects of atherosclerosis. One of the interesting properties of atherosclerotic plaques, but not of rat intimal thickening, is the pronounced thrombotic activity that can be observed when the luminal surface is disrupted. The reason for this response in far-advanced atherosclerotic plaques is not yet evident. Some investigators have proposed that the thrombotic activity is attributable to abnormal location and expression of two genes, tissue factor and plasminogen activator-1 (PAI-1), in the plaque. Because thrombosis is not a property of rat neointima, we are currently attempting to model human thrombosis by introducing smooth muscle cells that overexpress either tissue factor or PAI-1. The preliminary data support the conclusion that these genes may have a dramatic effect on local vascular thrombosis.

Local pharmacology

It would be highly desirable to control stenosis locally and avoid the systemic effects of the administered drug. We are currently using the smooth muscle cell–based gene transfer approach to develop pharmacologic strategies for controlling intimal hyperplasia and stenosis or restenosis after vascular surgery. In our initial experiments, we have focused on the role of matrix metalloproteinases in smooth muscle cell proliferation and migration and the development of the neointima. We have observed that two matrix metalloproteinases (72kD and 92kD gelatinases; MMP2 and 9) are expressed and active in the balloon-injured rat carotid artery. Although the systemic administration of drugs that affect the active site inhibits intimal thickening, these drugs have a toxic